

REMARKS

In response to the Office Action mailed September 6, 2007, Applicants have amended claims 1 and 25. No claims have been canceled and no new claims have been added. It is urged that support for all the above amendments may be found throughout the specification as originally filed, for example on page 5 and page 21, lines 15-19. No new matter has been added. The above amendments are not to be construed as acquiescence with regard to the Examiner's rejections and are made without prejudice to prosecution of any subject matter removed or modified by this amendment in a related divisional, continuation or continuation-in-part application. Following the amendments, claims 1, 5, 7, 10-13, 15, 16, 25, and 26 are pending in the application. Favorable reconsideration of the subject application is respectfully requested in view of the above amendments and the following remarks.

Objections to the Claims

The claims are objected to because they contain irregular spacing as a result of using the "justify" alignment tool. Applicant respectfully submits that these objections have been obviated by reformatting the claims using the "align left" setting, as recommended by the Examiner.

Applicant kindly requests that the Examiner withdraw these objections.

Claim Rejection under 35 U.S.C. § 112, first paragraph

Claims 1, 5, 7, 13, 15, 16, and 25 stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement for allegedly claiming subject matter which was not described in the specification in such a way as to reasonably convey to the skilled artisan that the inventor, at the time the application was filed, had possession of the claimed invention. Specifically, the Examiner asserts that Applicant's disclosure allegedly fails to define or describe a representative number of aminopeptidase substrates, wherein the aminopeptidase is substantially absent from at least one of the listed target microbes of claim 1.

Applicant respectively traverses this basis of rejection and submits that the skilled artisan would immediately understand that the inventor was in possession of the claimed species of aminopeptidase substrates at the time the application was filed.

Applicant submits and the Examiner has previously agreed that the as-filed specification provides numerous examples of aminopeptidase substrates and even L-alanine aminopeptidase substrates, and that the disclosed species are representative of aminopeptidase substrates in general. Applicant respectfully submits that aminopeptidases are exopeptidases, which cleave at the amino terminal end of a peptide chain to liberate a single amino acid, and thus, the skilled artisan would recognize that a defining characteristic of said substrate is the identity of the N-terminal amino acid that is specific for a given aminopeptidase. Moreover, the substrate for any given bacterial aminopeptidase is known in the art because there are only a finite number of amino acids, all of which are known. The skilled artisan would understand the inventor to be in possession of all such aminopeptidase substrates without actually knowing *a priori*, which specific aminopeptidase is absent from a target bacterium (*i.e.*, the aminopeptidase must have specificity for a known amino acid). Furthermore, all the substrates share a common structure, which is an amino acid or peptide (with a specific N-terminal amino acid) linked to a detectable signal moiety.

As an analogy, consider that you have a bag of M&Ms (the substrates known to the skilled artisan) consisting of all the colors of a rainbow. You have been shown examples of green, blue and yellow M&Ms. Because you can see the only difference in the M&Ms is the color (amino acid), you know that the remaining rainbow colored M&Ms have the same structure and merely consist of the remaining colors in the rainbow. Further consider that only people with a hair color matching a given M&M, may eat that M&M (specificity). If only a red haired person is missing from a room and everyone else in the room eats their respective M&Ms, you could you still describe the red M&Ms left in the bag, they still have the same specificity and structure as before and can only be selected from the finite number rainbow colors.

Applicants submit that the mere absence of a given aminopeptidase (*e.g.*, L-alanine aminopeptidase) in a target bacterium does not change the activity or specificity of that

aminopeptidase (L-alanine-reporter moiety), and thus, the substrate would unchanged and remain in possession of the skilled artisan.

Thus, the mere fact that an aminopeptidase is substantially absent from a target bacterium, does not take away from the fact that it has the same specificity for a member of a genus of finite and known amino acid based aminopeptidase substrates. Applicants submit that an aminopeptidase, which is substantially absent from a target bacterium does not mean that it has different enzymatic properties based on its absence. An L-alanine aminopeptidase would still have specificity for an N-terminal L-alanine amino acid linked to a reporter moiety, regardless of whether or not it is absent from a target bacterium.

Accordingly, Applicant respectfully submits that the as-filed specification amply describes a representative number of aminopeptidase substrates, all of which are known to the skilled artisan, because the identity of the amino acids for said substrate are already known. Accordingly, Applicant respectfully requests the Examiner carefully reconsider and withdraw this basis of rejection.

Claim Rejection under 35 U.S.C. § 112, first paragraph

Claims 1, 5, 7, 10-13, 15, 16, and 25 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. Specifically, the Examiner asserts that while the specification is enabling for production and use of compositions for identifying a pure culture sample of Gram-negative bacteria such as Campylobacter, said compositions do not reasonably provide enablement for the production and use of compositions for detecting any target microorganism in any sample, or even for detecting Campylobacter in a mixed sample, or for differentiating between Campylobacter and any Gram-positive bacteria.

Applicant respectfully traverses these bases for rejection and submits that the as-filed specification provides ample disclosure and guidance to practice the entire claimed scope of the invention.

The Examiner asserts that the presently claimed invention is not enabled for detecting Campylobacter in a mixed sample, or for differentiating between Campylobacter and any Gram-positive bacteria. Applicant submits that Examples I and II and Figure I clearly exemplify that the presently claimed invention can be used to reliably detect Campylobacter in a

sample of mixed bacteria (*i.e.*, poultry wash). In Example I, an aliquot of the sample is transferred to a growth-supporting medium for the target microorganism, namely *Campylobacter*. Applicant points out that the as-filed specification describes a particular embodiment on page 6, lines 3-8, wherein a growth medium supports the growth of target **or** non-target microorganisms (gram positive and gram negative) (emphasis added). A medium may specifically enrich the growth of *Campylobacter* (*i.e.*, the target microbe) and not other non-target microorganisms (p.21, lines 15-19). Formulations for such media are known in the microbiological arts and constitute one of the principle methods of isolating a particular strain of bacteria from a mixed sample. The growth supporting medium also contains a conditionally detectable marker, which reacts only with viable microorganisms (mostly *Campylobacter*) and an aminopeptidase substrate that cannot be enzymatically cleaved by the target microorganism, but instead can be processed by substantially all non-target microorganisms present in the sample (which are regarded as false positives). Applicant submits that Figure 1 provides evidence that the number of false positives in the poultry wash samples are negligible by demonstrating that the number of *Campylobacter* in said samples is the same, whether determined by the Applicant's method or the method approved by the U.S. government, wherein the presence of *Campylobacter* is confirmed by agglutination assays. Furthermore, Applicant has produced a commercial product for the specific detection of *Campylobacter* in mixed bacterial samples (see <http://www.biocontrols.com/products/simcamp.html>). Clearly, the presence of a functional commercial embodiment substantiates that no undue experimentation is required to practice the presently claimed invention. Thus, Applicants submit the presently claimed invention is enabled for identifying a pure culture sample of Gram-negative bacteria such as *Campylobacter*, detecting *Campylobacter* in a **mixed** sample, and for differentiating between *Campylobacter* and any Gram-positive bacteria (which may be among the small number of false positives).

The Examiner further contends that the skilled artisan would have to engage in undue experimentation to practice the invention with the additional target microorganisms recited in claim 1. Specifically, the Examiner contends that the as-filed specification does not provide examples of other aminopeptidases that are absent from the target microorganisms of claim 1. Applicant submits that claim 1 has been amended, without acquiescence, to recite the target microorganisms: *Salmonella*, *Listeria*, *E.coli* OH157, *Campylobacter*, and *Staphylococcus aureus*. Applicant submits that the skill in the microbiological arts is high, and as such, the

experimentation required to determine the “substantially absent” aminopeptidase is routine, and not complex. The skilled artisan in the microbiological arts routinely conducts high-throughput screens in order to classify organisms. Applicant submits the following references as evidence of the routine practice for determining aminopeptidase activities in bacteria: Peterson *et al.*, 1978. *Journal of Food Science*, 43 (6), 1853–1856.; Kampfer, 1992. *Journal of Clinical Microbiology*, Vol. 30, No. 5, p. 1067-1071.; and Westley *et al.*, 1967. *Applied Microbiology*, Vol. 15, No. 4, p. 822-825. Applicant submits that the skilled artisan would determine the “substantially absent” aminopeptidase from the target bacterium by performing aminopeptidase profiling of the sample to be tested (as only the aminopeptidase **activity** from the non-target bacteria in the type of sample to be tested is relevant, not the identity of the aminopeptidase itself) versus a pure culture of the target bacterium, and subsequently select the aminopeptidase activity that is substantially absent in the target bacterium and present in the remaining non-target bacteria in the sample. Applicant further submits that selective media were known in the art at the time of filing for the preferential growth of the target microorganisms in claim 1. Applicant cites Novier *et al.*, 2000. *Turk J Vet Anim Sci*, Vol. 24, p. 459-464 (*E. coli* OH157), Brackett *et al.*, 1989. *International Journal of Food Microbiology*, Vol. 8, p. 219-223 and Kovacs *et al.*, 1991. *Acta Microbiol Hung.* 38(2), pp.141-5 (*Listeria*), Aldridge *et al.*, 1977. *Journal of Clinical Microbiology*, Vol.6, No. 4, p.406-413 (*Staphylococcus aureus*), and Vassiliadis *et al.*, 1981. *Applied and Environmental Microbiology*, Vol.42, No. 4, p. 615-618 (*Salmonella*) in support that the skilled artisan had knowledge of the selective media necessary to perform the presently claimed invention in its full scope.

Applicant submits that the as-filed specification fully enables the skilled artisan to practice the invention in its full breadth of scope. The skilled artisan in the microbiological arts has experience in conducting high-throughput assays with regarding to aminopeptidase profiling (Peterson *et al.*, Kampfer, and Westley *et al.*). Furthermore, the skilled artisan is in the possession of the necessary growth specific media of the claimed target microbes of claim 1. Applicant submits that not only are the presently claimed methods enabled for the detection of *Campylobacter* as suggested by the Examiner, but for all claimed species in mixed bacterial samples employing the novel detection method of the Applicant.

Accordingly, Applicant kindly requests that the Examiner carefully reconsider and withdraw these bases for rejection.

Claim Rejection under 35 U.S.C. § 103(a)

Claims 1, 5, 7, 10-16 and 25-26 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Manafi *et al.* (J. Applied Bacteriology, 1990) in view of Molina *et al.* (Enfermedades Infecciosas y Microbiologia Clinica, 1991) and Tuompo *et al.* (U.S. Patent No. 5,420,017). Specifically, the Examiner contends that Manafi *et al.* disclose a method and composition for detecting Gram-negative bacteria in a sample using AAMC. Further, the Examiner contends that Tuompo *et al.* disclose a method and kit for detecting microorganisms in a sample using tetrazolium red. The Examiner alleges that the skilled artisan would find it obvious to combine the teachings of Manafi *et al.* with Tuompo *et al.* to arrive at the presently claimed composition. Applicant traverses these bases for rejection.

The Examiner alleges that claims 5 and 13 are not considered limitations of claim 1, and thus, the Examiner fails to give them any patentable weight. Applicant respectfully traverses this rejection and submits that the Examiner has mis-cited the MPEP §2111.02, which is directed at the meaning of a preamble within a claim. Applicant points out that claims 5 and 13 are dependent claims in which the preamble is “The composition of claim 1”, the transitional phrase is “wherein”, and the body of the claims specifying additional claim limitations are “said target microorganism is *Campylobacter*” (claim 5) and “wherein said non-target microorganisms include substantially all non-*Campylobacter* species” (claim 13). Applicant respectfully submits that patentable weight should be given to dependent claims, which clearly alter the structure (*i.e.*, restricting the scope of claim 1 would restrict the reagents used to practice the invention, specific media, substrates, *etc.*) and scope of the claim from which they depend. For example, these claims substantially narrow the scope of target and non-target microorganisms.

Further, the Examiner alleges that the skilled artisan would find it *prima facie* obvious to modify the composition of Manafi *et al.*, which includes AAMC in an agar plate, to further include tetrazolium red, as taught by Tuompo *et al.* Applicant submits that all bacterial colonies plated grow on the agar plate of Manafi, because the plate is non-selective (*e.g.*, Plate Count Agar was used). Applicant submits a positive tetrazolium red signal would be present for

all the colonies on the plate, and thus, give no additional information. As the plate includes AAMC, Gram-negative colonies on the sample will produce a positive signal for AAMC fluorescence, and thus, most of the remaining, non-flourescent colonies on the plate would be Gram-positive or *Campylobacter* bacterial colonies (*i.e.*, the major groups of bacteria that cannot hydrolyze the AAMC substrate). AAMC is not selective for viability, it is a molecule specifically cleaved by L-alanine aminopeptidase organisms, but does not indicate viability. Further, the non-flourescent, Gram-positive and *Campylobacter* colonies of Manafi *et al.* are not discernable from one another, because the medium used by Manafi *et al.* does not selectively support the growth of the target microorganism. In contrast, the Applicant's media, which selectively supports the growth of the target microorganism and not the non-target microorganism, can accurately discriminate between Gram-positive, Gram negative and *Campylobacter* colonies (see Examples I and II and Figure 1).

The Examiner contends that including the viability marker in the agar plate of Manafi *et al.* would provide an extra measure of quality control in the methods of Manafi *et al.* as the viability marker would provide a positive reading, indicating the bacteria were successfully transferred in a viable state to the agar plates. Applicant submits that this line of reasoning is faulty because on a non-selective medium, only viable colonies will grow and they will grow both before and after transfer.

The Examiner further contends that if the sample of Manafi *et al.* is L-alanine aminopeptidase negative, no signal is produced, yet there is no way to determine if the sample is merely negative for L-alanine aminopeptidase or if the sample was not successfully plated. Applicants submit that the Examiner's reasoning is not clear. Because the medium of Manafi *et al.* is not selective, there are basically only 4 outcomes:

- 1) no colonies on the plate, no fluorescence, which is equivalent to a faulty plating;
- 2) colonies on the plate, no fluorescence, which is equivalent to having either Gram-positive or *Campylobacter* colonies (*i.e.*, non-fluorescent because they lack L-alanine aminopeptidase);
- 3) colonies on the plate, all fluorescence, which is equivalent to having fluorescent Gram-negative colonies except *Campylobacter*; or
- 4) a combination of 2, and 3.

Applicant submits there would never be a case where one could prove that there was no fluorescence from a non-viable colony (a faulty plating) because non-viable colonies don't grow and are not detectable by tetrazolium red or any other means.

Further, the Examiner maintains that including the viability marker tetrazolium red would provide an extra measure to ensure the sample was transferred successfully and that a false negative was not obtained due to a dead sample. As mentioned above, if the sample of Manafi *et al.* is not plated successfully, no colonies will grow, and there will be no fluorescence. Applicant submits that a true positive for the embodiment contemplated by the Examiner is a Gram-negative bacterium that is not *Campylobacter* (*i.e.*, AAMC+, and forms a colony), and thus, a true negative would be a Gram positive or *Campylobacter* bacterium (*i.e.*, AAMC-, and forms a colony). In this case, a false negative would comprise an AAMC- colony that is formed by a Gram-negative bacterium (other than *Campylobacter*). Thus, a false negative in the embodiment contrived by the Examiner, in view of Manafi *et al.*, is a non-*Campylobacter*, Gram-negative bacterial colony that lacks sufficient L-alanine aminopeptidase activity to hydrolyze AAMC. Applicant submits that tetrazolium red cannot discriminate between this false negative colony and a dead colony, because dead colonies do not grow, and thus, are not detectable. Moreover, addition of tetrazolium red adds nothing to the invention of Manafi *et al.* because the skilled artisan can only evaluate colonies that are either fluorescent or non-fluorescent (*i.e.*, he cannot evaluate a "dead sample" because there is nothing to evaluate); and to form a colony you must be a viable bacterium; and all viable bacteria are positive for tetrazolium red. Thus, all colonies, which comprise both Gram-negative and Gram-positive bacteria, on the plate of Manafi *et al.* are labeled with tetrazolium red. Moreover, Applicant questions the relevance of basing an obviousness rejection on the premise that the combination aids in the detection of a false negative, wherein false negatives are not the target bacterium, which is a pathogen

In summary, neither Manafi *et al.*, Molina *et al.*, or Tuompo *et al.* alone or in combination, can detect a target microorganism from a mixed sample of microorganisms based on a positive signal from a viability marker and the absence of signal from an aminopeptidase substrate, wherein the target bacterium lacks sufficient aminopeptidase activity specific to hydrolyze said substrate. Applicant uses a growth supporting media for the target microorganism, which selectively enriches for growth of the target microorganism over the non-

target microorganism (see Example I and II, and Figure I). Moreover, such media are known in the art and exist for all the claimed microorganisms of claim 1. Applicant submits that most bacteria growing on the “plate” should be target bacteria because the medium is growth supporting for the target microorganism. It logically follows that most viable colonies (*e.g.*, tetrazolium red positive) are target bacteria, but that there are some non-target bacteria that may grow and would constitute false positives at this level (see <http://www.biocontrols.com/products/simcamp.html>). In order to discriminate between the false positives and true positives, Applicant includes an unobvious second layer of screening for false positives, by assaying for cleavage of a false positive specific substrate (*i.e.*, a substrate that cannot be cleaved by the target microorganism). Thus, the result of the Applicant’s assay, is a “plate” of colonies comprising mostly target bacteria, all of which stain positive for the viability marker, but for which only the few false positives give a positive signal for the cleavage of the aminopeptidase substrate that cannot be cleaved by the target microorganism. As described in detail above, the medium of Manafi *et al.* is non-selective, and Tuompo *et al.* does not remedy this insufficiency. Moreover, the combination of the inventions of Manafi *et al.* and Tuompo *et al.* would never result in the presently claimed compositions. Thus, Applicant submits that the skilled artisan would not find it *prima facie* obvious to combine a viability marker with an invention that only detects viable microorganisms (*i.e.*, the combination serves no purpose).

Applicant submits the references cited by the Examiner fail to establish a *prima facie* case of obviousness against the claimed invention, and requests that the Examiner carefully reconsider and withdraw this basis for rejection.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

All of the claims remaining in the application are now believed to be allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,
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